ON THE TOXIC EFFECT OF DILUTE SOLUTIONS OF ACIDS AND SALTS UPON PLANTS.

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(WITH PLATE VII)

I. Introduction.

The desire for a deeper and clearer insight into the subject of nutrition of plants has led many botanical investigators to endeavor to determine the poisonous or nutritive qualities of a large number of compounds. Within the last decade a considerable number of papers have appeared dealing with the toxic effect of compounds which are generally classed as non-nutritive. The majority of these older experiments have been along the same line, and so far as known the compounds have been prepared by making solutions of a certain per cent. by weight. During the last year Kahlenberg and True[1] have carried out numerous experiments with very many inorganic and organic acids and various salts in which a different method was employed. In these experiments the solutions were prepared according to gram equivalents, and the results obtained are extremely interesting both from the chemical and the biological point of view. All of the experiments alluded to were carried out with a single plant, *Lupinus albus*.

It is important to know whether these results can be confirmed by the use of other plants, which might be expected to differ in their reactions; and to this end, at the suggestion of Dr. Kahlenberg, the present investigation has been made.

Before taking up the results in detail it may be well to present a few general considerations in regard to plant, or rather protoplasmic, poisons. Compounds which have a toxic effect upon animals are generally poisonous to plants, although we find different degrees of sensibility to the same compound in

[1] Bot. Gaz. **22**: 81. 1896.

both plants and animals. The toxic effect of a substance is evidently due to one of two things. In plant tissue the effect upon the *turgescence* of the cell must play an important part,[2] since when the turgor-pressure is suddenly and decidedly decreased, the growth is either retarded or inhibited. An inhibition or a retardation of growth must then be regarded as a symptom of poisoning. In comparison with turgescence the *direct effect* upon the protoplasm is by far the more important, since in simple turgor experiments the retardation of growth is due to the irritation of the protoplasm as well as to the turgor-change. Now, since the irritability of the protoplasm of different plants differs widely, we may reasonably expect quite a wide range in the amount of different compounds which various plants will withstand.

Why and how certain substances have a toxic effect, and certain others a nutritive value, is not known. For example, it is at present impossible to say why it is that potassium sulfate has a nutritive value while copper sulfate produces a toxic effect. Those substances which are poisonous to plants are generally such substances as are not accessible to plants in their normal habitats, at least to any extent, while those substances which are generally present in the soil have no injurious effect, or at least not in the same degree of concentration at which we find them in the soil. If the poisonous action of various substances is a mere matter of adaptation and adjustment, as seems to me highly probable, then we might expect that by adding gradually more and more copper sulfate to the soil in which a plant is growing it would come in the course of time to adapt itself to quite a large amount of this salt, which is normally extremely poisonous to the majority of plants. So far as known no experiments of exactly this nature have been carried out, and an experimental test of this would be highly interesting.

2. METHOD.

In the experiments performed, three different plants were used: *Pisum sativum*, *Zea Mais*, and *Cucurbita Pepo*. I describe

[2] Annals of Botany 9 : 385. 1895.

first the method of germination. The peas were placed in a beaker, covered with distilled water and allowed to soak for twenty-four hours; they were then placed in a Petri-dish between moistened sheets of filter paper and allowed to remain until the radicles just burst through the seed coats. In all the experiments it was important that the seedlings should have straight roots, since it was necessary to measure the roots at intervals to determine the growth. If the peas were allowed to remain between sheets of moistened filter paper the roots grew crooked and twisted and could not be used, so it became necessary to resort to other methods of growing them.

Two different methods were used, both of which were quite satisfactory.

1. A thin sheet of cork was perforated by means of a cork-borer with a series of holes as shown in the diagram (*fig. 1*), the large opening being just small enough to keep the pea from slipping through. This cork was then floated in a deep Petri-dish of distilled water, and as soon as the radicle had burst through the seed coats the peas were transferred to the cork. The peas were so placed that the radicle was directed toward the small opening of the cork, and the whole was covered with a sheet of filter paper which hung down into the water and thus kept the peas moist (*fig. 2*). The peas were then allowed to grow until they were of sufficient size for use in the experiments. In cases where germination was slow it was found necessary to change the distilled water several times before the seedlings were of sufficient size for use. In the majority of seedlings the roots grew quite straight.

2. Another method, quite similar to the above, was also used. Two sheets of cork were taken; one was provided with openings somewhat larger than the pea; the other sheet of cork was provided with smaller openings which would register as shown in diagram when the two corks were placed one above the other (*fig. 3*). The two sheets of cork were then wired together and the small openings were provided with glass tubes about 5^{cm} long. The whole was placed in a deep dish and the peas were

placed in the openings in such a manner that the roots grew down the glass tubes (*fig. 4*). By this method it was impossible for the roots to grow crooked.

The seedlings of *Zea Mais* and *Cucurbita Pepo* were grown in a different way since germination took place more readily, and there was not so great a tendency to grow crooked roots. The seeds were soaked in distilled water for twenty-four hours, and then placed carefully between sheets of moistened filter paper. Care was taken to place the seeds in a flat position and in most cases the roots were straight, so that the extra work of a transfer was avoided. In all cases the seedlings were allowed to grow until the roots had attained a length of about twenty millimeters. For the acids, *normal* stock solutions[3] were used, that is, solutions of the mono-basic acids like HCl would contain one gram-molecule of HCl per liter, while the di-basic acid like H_2SO_4 would contain one-half gram-molecule of H_2SO_4 per liter, so that in each case the normal solution would contain one gram of H per liter. For the stock solutions of the various salts, either solutions containing a gram-molecule per liter were used or solutions containing a certain fraction of a gram-molecule. For the seedlings of *Pisum sativum* the experiments were carried out in the following manner: The solutions in which the seedlings were to be grown were placed in small beakers of about 300^{cc} capacity. A cork of sufficient size to close the opening of the beaker was provided with a glass rod which extended down into the solution. On the end of the glass rod was a smaller cork, and the seedlings were supported on this cork by means of glass pins. The cork was then set at such a level that the cotyledons of the seedlings were just above the surface of the solution. This will also be made plain by the appended diagram (*fig. 5*).

For the seedlings of *Zea Mais* and *Cucurbita Pepo* a more convenient method was found. A small piece of sheet cork of sufficient size to float in the beaker was provided with two openings and the seedlings were placed in these so that the roots

[3] Special thanks are due to Dr. Kahlenberg for the majority of the stock-solutions, and also to Mr. Schlundt for several salt-solutions.

were immersed in the solution, and the beaker covered by a cork to prevent evaporation (*fig. 6*).

In all of the experiments performed two seedlings were used. Before placing them in the solutions they were allowed to grow until the roots had reached a length of about 20^{mm}, and then a distance of 15^{mm} was marked off from the tip of each root by means of a fine brush and India ink. The time was then recorded, and at twenty-four hours from that time, the seedlings were removed from the solutions and measured again and the growth recorded. The roots were also carefully examined for any other symptoms of poisoning besides the retardation or inhibition of growth. They were then replaced in the solutions and allowed to stand for another twenty-four hours when measurements were again made. In all cases the seedlings were grown in a dark chamber with nearly a uniform temperature (21°–23° C.) The lengths given in the tables are the average growth per twenty-four hours.

The mixture of the solutions for the growth of seedlings was made as follows:

10^{cc} of nor. sol. to $1000^{cc} = \frac{N}{100}$
25^{cc} of $\frac{N}{100}$ to $200^{cc} = \frac{N}{800}$
100^{cc} of $\frac{N}{800}$ to $200^{cc} = \frac{N}{1600}$
100^{cc} of $\frac{N}{1600}$ to $200^{cc} = \frac{N}{3200}$ etc.

3. H-ACIDS.

Two seedlings of *Pisum sativum* were placed in each of the solutions of the strengths shown in the table and the growth recorded for forty-eight hours.

Acids.		$\frac{N}{1600}$	$\frac{N}{3200}$	$\frac{N}{6400}$	$\frac{N}{12800}$
HCl	1st 24 hours...	.....	.....	3.25^{mm}	4.75^{mm}
	2d 24 hours...	.....	.....	.....	7.5 "
H_2SO_4	1st 24 hours...	.....	.....	3.5 "	12.5 "
	2d 24 hours...	.....	.....	.....	5.25 "
HNO_3	1st 24 hours...	.....	.....	.....	6.5 "
	2d 24 hours...	.....	.....	.....	7. "
HBr	1st 24 hours...	.....	.....	.....	5.5 "
	2d 24 hours...	.....	.....	.....	6.25 "

In the $\frac{N}{1600}$ and $\frac{N}{3200}$ solutions no growth whatever occurred, and at the end of the first twenty-four hours the tips of the roots were very soft and flabby and were considered as dead. In the $\frac{N}{6400}$ solution a growth occurred in the HCl and H_2SO_4 solutions for the first twenty-four hours, but the second twenty-four hours showed no additional growth, while the roots were very soft and flabby. In the HNO_3 and HBr no growth whatever took place in the $\frac{N}{6400}$ solution. In the $\frac{N}{12800}$ solution the seedlings grew for the entire forty-eight hours and at the end of that time the roots were very rigid and did not show any symptoms of poisoning.

It is worthy of note here that in case of the seedlings in the $\frac{N}{6400}$ solution a large number of lateral roots were formed before the root was killed. The delicate cells of the root tip were the first to be affected by the poison, and thus having its main growing point destroyed the plant was stimulated to the production of lateral roots in its struggle to withstand the effects of the poison. Another point which cannot be overlooked is the fact that in the $\frac{N}{1600}$ solution of HCl the roots of the seedlings were covered with a dense growth of fungus at the end of the experiment. The species of the fungus was not determined. So far as known from experiments it is true that fungi generally are able to withstand stronger solutions of poison than green plants. *Penicillium*, for example, will grow in a comparatively strong solution of $CuSO_4$. A solution of $\frac{N}{100}$ acetic acid, after standing for some time in the laboratory, was found to be filled with a dense growth of fungus, the species of which was not determined.

Two seedlings of *Zea Mais* were placed in each of the solutions recorded in the following table, and the growth recorded at periods of twenty-four hours.

From the degree of concentration at which the seedlings of *Pisum sativum* were killed it was thought that the solutions $\frac{N}{3200}$, $\frac{N}{6400}$, and $\frac{N}{12800}$ ought to show the strength of solution which these seedlings would withstand, and so at first only solutions of that strength were used. In all of these, however, the

growth was considerable for the first twenty-four hours, with generally an increase in the amount of growth for the next twenty-four hours. A glance at the following table will also show that the growth in the $\frac{N}{3200}$ and $\frac{N}{6400}$ solutions was somewhat less than in the $\frac{N}{12800}$ solution, so that even if the growth was not inhibited in the former a very perceptible retardation of the growth occurred. Since growth was not entirely inhibited in the solutions from $\frac{N}{3200}$ upward, seedlings were placed in two stronger solutions, $\frac{N}{800}$ and $\frac{N}{1600}$. In the $\frac{N}{800}$ solution no growth whatever occurred, and at the end of the first twenty-four hours the roots were very soft and flabby near the tip. In the $\frac{N}{1600}$ solution, however, the roots showed quite an increase in length for the first twenty-four hours but for the second twenty-four hours showed no increase, so that the $\frac{N}{1600}$ solution is the strength at which the roots were killed.

ZEA MAIS.

Acids.		$\frac{N}{800}$	$\frac{N}{1600}$	$\frac{N}{3200}$	$\frac{N}{6400}$	$\frac{N}{12800}$
HCl	1st 24 hours	..	3.5 mm	8.25 mm	11.5 mm	11.25 mm
	2d 24 hours	..	.....	18.25 "	16 "	15.25 "
H_2SO_4	1st 24 hours	..	2.75 "	12.5 "	11.5 "	17.5 "
	2d 24 hours	..	.....	27.5 "	14 "	33 "
HNO_3	1st 24 hours	..	4.5 "	7 "	14 "	16.5 "
	2d 24 hours	..	.5 "	9 "	11.5 "	29 "
HBr	1st 24 hours	..	3 "	7.5 "	12 "	15 "
	2d 24 hours	..	.....	8.25 "	13.75 "	30.5 "

A glance at the table for *Pisum sativum* shows a very considerable difference in the amount of the acids which the seedlings could withstand. In the case of *Pisum sativum* the $\frac{N}{6400}$ solution was of sufficient strength to inhibit the growth, while in the case of the *Zea Mais* seedlings it required a $\frac{N}{1600}$ solution, or a solution four times as concentrated. This very great difference in the degree of irritability is the more worthy of note, since the one, *Pisum*, has its reserve food supply stored in the form of carbohydrates, while corn contains quite a large amount of fatty material.

Two seedlings of *Cucurbita Pepo* were placed in each of the solutions of the strength shown in the following table:

CUCURBITA PEPO.

Acids.		$\frac{N}{1600}$	$\frac{N}{3200}$	$\frac{N}{6400}$	$\frac{N}{12800}$
H Cl	1st 24 hours...	.....	.....	2.5 mm	3.75 mm
	2d 24 hours...	.....	.....	3.25 "	9.25 "
H_2SO_4	1st 24 hours...	.....	.....	9. "	6. "
	2d 24 hours...	.....	.....	4.75 "	6.5 "
H NO_3	1st 24 hours...	.....	.....	5.25 "	3.5 "
	2d 24 hours...	.....	.....	8. "	4. "
H Br	1st 24 hours...	.....	.....	4.5 "	5.75 "
	2d 24 hours...	.....	.....	2. "	1.75 "

Seedlings were set first in the $\frac{N}{1600}$ and $\frac{N}{3200}$ solutions and after the first twenty-four hours no growth had taken place and the root tips were soft and flabby; they were, however, replaced in the solutions and allowed to stand for another twenty-four hours. At the end of the forty-eight hours no additional growth had occurred. In the $\frac{N}{6400}$ and $\frac{N}{12800}$ solutions the growth was considerable for both the first and second twenty-four hours. Then the strength of solution necessary to inhibit the growth is $-\frac{N}{3200}$, which is less than in the case of *Pisum sativum* seedlings, but more than in the case of *Zea Mais* seedlings.

The relative sensibility to the acid poisons then is as follows:

1. Pisum sativum, seedlings killed by $\frac{N}{6400}$ solution.
2. Zea Mais, seedlings killed by $\frac{N}{1600}$ solution.
3. Cucurbita Pepo, seedlings killed by $\frac{N}{3200}$ solution.

Before discussing the results of the experiments with the acids, a short statement in regard to the so-called theory of *electrolytic dissociation* will be necessary. The theory was published by Arrhenius[4] in 1887 and amounts practically to this: Aqueous solutions of acids, bases, or salts are, to a greater or less extent, broken up or dissociated into part-molecules, the so-called *ions*. It is not necessary to mention here the facts which confirm this theory, but it suffices to say that it now stands on a

[4] Zeitschrift für Physikalische Chemie 1: 631.

comparatively firm experimental basis. The amount of dissociation depends upon the strength of the solution. The more dilute the solution, the more complete is the dissociation, until at infinite dilution the dissociation is complete. When a certain acid, for example HCl, dissociates, the result is H-ions and Cl-ions; the H-ions are charged positively with electricity, while the Cl-ions are charged negatively, there being an equal number of positive and negative ions in order to preserve equilibrium. The manner of dissociation may be expressed by H^+ and Cl^-. In the case of a salt, as $CuSO_4$, for example, the dissociation will take place as $^+Cu^+$ ions and $^-SO_4^-$ ions, and in a similar manner for other salts, the radicle always being the electro-negative *ion* and the basic element or radicle the electro-positive ion.

A comparison of the results obtained with the acids, with some investigations on the plant cell, is interesting as affording some light upon the nature of the effect produced by the acids. Klemm[5] states that ½ to 1 pro mille HNO_3 causes the streaming motion of the protoplasm in the hairs of *Trianea* to cease and also produces a granulation and aggregation of the protoplasm. A 1 promille solution of HNO_3 would contain 1 gram of HNO_3 to 1000^{cc} of water. The strength of solution which killed the roots of *Pisum sativum* was $\frac{N}{6400}$, which is equivalent to 1 gram of HNO_3 to 101,587^{cc} of water. For *Zea Mais* roots the killing point was the $\frac{N}{1600}$ solution, which is equivalent to 1 gram of HNO_3 to 25,396^{cc} of water. For the *Cucurbita Pepo* seedlings the roots were killed by the $\frac{N}{3200}$ solution, which is equivalent to 1 gram of HNO_3 to 50,793^{cc} of water. From these figures it will be seen that the strength which was required to produce a disorganization of the protoplasm in the *Trianea* hairs was so much greater than that required to kill the roots of the seedlings, that the toxic effect can hardly be due to a visible disorganization of the protoplasm. Klemm also states that the same thing takes place with equally dilute solutions of H_2SO_4 and HCl.

[5] Desorganisationserscheinungen der Zelle; Jahrbücher f. wiss. Botanik **28**: 658–964. 1895.

In regard to the relation of these results to the theory of dissociation, Kahlenberg and True[6] have demonstrated quite clearly in their work that it is the H^+ ion which produces the toxic effect. HCl will form H^+ ions and Cl^- ions; H_2SO_4 will dissociate first into H^+ and HSO_4^-, but the final product will be two H^+ ions and $^-SO_4^-$ ions; HNO_3 splits to form H^+ ions and NO_3^- ions; HBr will dissociate to form H^+ ions and Br^+ ions. As has been before stated, the more dilute the solution, the more complete the dissociation, but with the dilutions used for the acids, dissociation would be practically complete, so that we need not take into consideration anything but the H^+ ions and the electro-negative ions. Take for example NaCl, which will dissociate as Na^+ and Cl^- ions. Now NaCl at the dilution at which the HCl was effective is practically without effect; the Cl^- ions must then be considered as non-poisonous in the HCl, since both HCl and NaCl contain Cl^- ions. Now if the Cl^- ions are without any toxic effect at this dilution it is plain that the poisonous effect must be due to the H^+ ions.

The H_2SO_4 may be considered in the same way. If a plant be subjected to an equally concentrated solution of K_2SO_4, which is one of the compounds from which plants quite commonly obtain their potassium, it would be entirely unharmed at that dilution. The K_2SO_4 would dissociate to form K^+ ions and SO_4^- ions, and since K_2SO_4 and H_2SO_4 solutions have SO_4^- ions in common it is evident that the SO_4^- ions of the H_2SO_4 are non-poisonous. This then leaves only the H^+ ions to produce the toxic effect. The non-poisonous character of the SO_4^- ions can be shown by the action of other sulfates, such as $CaSO_4$, $MgSO_4$ and Na_2SO_4. Again: sulfur is a constant constituent of proteid substances, and is absorbed by all plants in the form of sulfates, so that plants are constantly subjected to the action of SO_4^- ions. The solutions were so mixed that the H_2SO_4 solutions contained the same amount of ionic H as the other acids, that is, a normal solution of H_2SO_4 contained only ½

[6] BOT. GAZ. 22:81. 1896.

gram molecule to the liter. Now the H_2SO_4 solution which contains the same amount of ionic H as the other acids kills at the same point of dilution, so that this again points to the toxic effect of the H^+ ions.

The action of HNO_3 may be discussed in a similar manner. $Ca(NO_3)_2$ is one of the common compounds by which a plant receives calcium and nitrogen and at a dilution relatively the same as that at which the acid killed the seedlings, it is without any harmful effect. It is presented to the plant in the form of $^+Ca^+$ ions and NO_3^- ions. The HNO_3 and $Ca(NO_3)_2$ contain NO_3 in common and since the NO^-_3 ions are non-poisonous it leaves the H^+ ions again as the agent which produces the toxic effect. A large part of the nitrogen contained in a plant is supplied to it in the form of nitrates, so that here again the plant is constantly subjected to the action of NO^-_3 ions. The non-poisonous character of the NO^-_3 ions may be shown by other nitrates as well.

It may seem doubtful at first whether HBr can be considered in the same way as the other acids, but Dirck[7] has found that KBr in dilute solution produces no harmful effect. Now since this would dissociate as K^+ ions and Br^- ions, it follows that in dilute solution Br-ions are non-poisonous, and hence play no part in the toxic effect of the HBr, at least not at the dilution at which the HBr killed the roots of seedlings. It will also be seen from the tables that the HBr kills the seedlings at the same degree of concentration as the other acids; now since it has been shown that the toxic effect of HCl, H_2SO_4 and HNO_3 is due to the H^- ions, we should expect HBr to kill at a different degree of concentration if the Br was poisonous also, for then we should have the sum of the effect of H^+ ions and Br^- ions. Here again the entire toxic action is produced by the H^+ ions.

It has been clearly shown by the above experiments that in the case of poisoning by acids, the harmful effect is produced entirely by the H^+ ions. By putting the results of the experiments in a different form we can get a better idea of the

[7] Bericht d. Verhdlg. d. sächs. Ges. d. Wiss. zu Leipzig **21**: 20. 1869.

extremely small amount of ionic H necessary to kill the roots of seedlings. In the case of the *Pisum sativum* seedlings one part of ionic H^- to 6,400,000 of water was sufficient to kill the roots. The roots of *Cucurbita Pepo* were killed by 1 part of ionic H^- to 3,200,000 parts of water, while the roots of *Zea Mais* were the most resistant, requiring one part of ionic H to 1,600,000 parts of water.

When expressed in the form of per cent. the extremely small amount of acid necessary to kill the *Pisum sativum* seedlings is even more apparent, and may be expressed as follows: HCl, 0.00056%; H_2SO_4, 0.00076%; HNO_3, 0.00098%; HBr, 0.00126%.

From this it will be seen that the per cent. according to weight gives a different result, showing apparently a difference in the toxic power of the acids, which would be obtained if the theory of dissociation was overlooked.

Before leaving the subject of acids and their toxic effect a few points in connection with the relation of the plant to CO_2 are worthy of note. All CO_2 which reaches the plant, whether it be the root or the green aerial parts must be brought into solution before it can be absorbed. As soon as CO_2 and water come together we no longer have simple CO_2 and water but H_2CO_3, carbonic acid. Now this carbonic acid in aqueous solution will dissociate to form two H^+ ions and $^-CO_2^-$ ions. CO_2 has been found to be poisonous to the green parts of plants, and the question which naturally suggests itself is, does the toxic effect depend simply upon the CO_2, as has always been stated, or does the ionic H of the carbonic acid bring about the poisoning? In the case of the green parts of a plant the amount of CO_2 necessary to produce a toxic effect is quite large, and this may be urged as an objection to the ionic explanation. This can however be easily explained. The CO_2 absorbed by a leaf is taken up with some difficulty, the resistance depending upon the structural porosity of the leaf and upon the permeability of the cell walls, so that it takes a very considerable external pressure or a large per cent. of CO_2 in the surrounding air to cause the

accumulation of CO_2 to any amount in the tissue of the plant since it is constantly being removed from the scenes of activity in the photosyntactic processes.

That the ionic explanation is the true one is also strengthened by the experiments of Gigliole,[8] who found that various seeds when subjected to the action of CO_2 in their dry condition, retained their vitality as well as in ordinary air, but when the seeds were soaked in water they were killed. This effect seems to me to be due to the H-ions present.

The experiments in regard to the poisonous action of CO_2 are somewhat conflicting since Jentys[9] concluded from a series of experiments that beans, lupines, rye, and wheat were not harmed by CO_2. These experiments, however, are not conclusive, since the plants were grown in earth in glass pots. The CO_2 was introduced by a tube in the floor of the pot, and since the air which was passed in contained only about $\frac{4}{12}$ per cent. CO_2 the roots were probably subjected to only a small per cent. of CO_2 in the form of carbonic acid. In the experiments which I have performed with the acids the whole plant was not killed but simply the main root, so that if the plants had been growing in the soil they would not have been killed. Fungi are also able to withstand more CO_2 than green plants, which has been shown to be the case with other acids. More experiments are necessary to prove conclusively the fact that CO_2 poisoning is due to the effect of the ionic H^-, and as soon as possible experiments with that view will be carried out.

ACETIC ACID.

$CH_3 COOH$		$\frac{N}{400}$	$\frac{N}{800}$	$\frac{N}{1600}$	$\frac{N}{3200}$	$\frac{N}{6400}$
P. sativum	1st 24 hrs	....	.....	.....	4.75 mm	1.25 mm
	2d 24 hrs	....	.....	.....	5.25 "	14.5 "
Zea Mais	1st 24 hrs	....	10.25 mm	15.5 mm	11 "	.....
	2d 24 hrs	....	6.75 "	10.5 "	27 "	.....

[8] Gazetta chimica italiana 9: 477–478. 1879.

[9] Extrait du Bulletin de l'Académie des Sciences de Cracovie, July 1892.

Some experiments with acetic acid also were performed. In these, only seedlings of *Pisum sativum* and *Zea Mais* were used, and the results at the different dilutions are shown in the preceding table.

From the above table it will be seen that $\frac{N}{1600}$, $\frac{N}{3200}$ and $\frac{N}{6400}$ solutions were used for the seedlings of *P. sativum*. In the $\frac{N}{1600}$ solution no growth whatever occurred and at the end of the first twenty-four hours the root tips were very soft and flabby. In the two remaining solutions considerable growth resulted, but it will be seen from the amount of growth that the $\frac{N}{3200}$ solution produced a marked retardation.

For the *Zea Mais* seedlings much greater concentrations were used to start with, since it had been found in the experiments with other acids that these seedlings were much more resistant. In the $\frac{N}{400}$ solution no growth whatever occurred while in the other dilutions the growth was considerable. The figures here also show that growth was to some extent retarded in the dilutions which were not sufficient to kill the roots.

The dissociation of acetic acid is into H^+ ions and $C_2H_3O_2^-$ ions, so that here again we have to deal with H^+ ions. In this acid, however, the degree of dissociation is not complete at the dilutions which were used in the experiments. The amount of dissociation in a $\frac{N}{1024}$ solution is only 12.66 per cent. according to Ostwald,[10] and in a $\frac{N}{512}$ solution 9.14 per cent. Then here we have to deal with a certain amount of dissociated acetic acid and a certain amount which remains unchanged, the effect of which cannot be overlooked. Sodium acetate in equally strong solution is non-poisonous and since both sodium acetate and acetic acid contain $C_2H_3O_2^-$ ions in common it follows that the $C_2H_3O_2^-$ ion is non-poisonous, so that the H^+ ions and the undissociated acid are left to produce the toxic effect. The part which is played by these two will depend upon the degree of concentration of the solution.

It will be noted that in the case of both seedlings the per cent. necessary to kill the roots is much greater than in the

[10] Zeitschrift für Physikalische Chemie 3: 174.

experiments upon other acids. This fact is to be explained by the partial dissociation of the acetic acid.

It is also interesting to note that after the killing point has been found for the *Pisum sativum* roots it can be worked out by proportion for the *Zea Mais*. $\frac{N}{6400}$, the killing point in other acids for *Pisum*, is to $\frac{N}{1600}$, the killing point for *Zea Mais* with other acids, as $\frac{N}{1600}$, the killing point of acetic acid for *Pisum sativum*, is to $\frac{N}{x}$, the killing point for *Zea Mais*, that is,

$$6400 : 1600 :: 1600 : x;$$

whence $x = 400$, and a glance at the table will show that the $\frac{N}{400}$ solution was sufficient to kill the roots of *Zea Mais* seedlings.

Klemm [11] has shown that more concentrated solutions of the organic acids are necessary to produce disorganization than of the inorganic acids. This fact then falls in line with the result here obtained for the acetic acid.

4. Copper Salts.

For the copper salts stock solutions which contained $\frac{1}{160}$ gram-molecule to the liter were used. Three different copper-salts were used, copper sulfate, copper chloride, and copper acetate, and the dilutions were made as follows:

10^{cc} of $\frac{1}{160}$ to 200^{cc} = $\frac{1}{3200}$ mol.
100^{cc} of $\frac{1}{3200}$ to 200^{cc} = $\frac{1}{6400}$ mol.
100^{cc} of $\frac{1}{6400}$ to 200^{cc} = $\frac{1}{12800}$ mol. *etc.*

PISUM SATIVUM.

Copper salts.	$\frac{1}{6400}$ mol.	$\frac{1}{12800}$ mol.	$\frac{1}{25600}$ mol.	$\frac{1}{51200}$ mol.	$\frac{1}{102400}$ mol.
$CuSO_4$ 1st 24 hours	....	....	0.75 mm	2.5 mm	14 mm
$CuSO_4$ 2d 24 hours	....	....	.....	... .	13.75 "
$CuCl_2$ 1st 24 hours	....	....	0.75 "	3.25 "	15 "
$CuCl_2$ 2d 24 hours	....	....	.....	3 "	8.25 "
$Cu(C_2H_3O_2)_2$ 1, 24 h	....	....	0.75 "	3.5 "	12.75 "
$Cu(C_2H_3O_2)_2$ 2, 24 h	....	....	.....	3.5 "	9 "

Experiments were carried out first with the seedlings of *Pisum sativum*, two seedlings being placed in each of the solu-

[11] Jahrbücher f. wiss. Botanik **28**: 658–664.

tions indicated in the preceding table. The amount of growth for periods of twenty-four hours was noted.

In the solutions which contained $\frac{1}{6400}$ and $\frac{1}{12800}$ molecule per liter no growth whatever was observed, and at the end of the experiment the root tips were generally quite soft and flexible and in most cases showed a faint greenish coloration. In the next dilution, $\frac{1}{25600}$ molecule, a very slight growth was observed for the first twenty-four hours but for the second twenty-four hours no growth whatever, and at the end of the experiment these roots were also soft and flexible and colored greenish. In the two next dilutions $\frac{1}{51200}$ molecule and $\frac{1}{102400}$ molecule, the growth was considerable, but it was much greater in the later solution showing that the growth was retarded to a considerable extent by the $\frac{1}{51200}$ molecule solution. In the $\frac{1}{51200}$ molecule, $CuSO_4$ solution no growth resulted for the second twenty-four hours, but this is not strange since in the others the growth was retarded to a considerable extent. Then the $\frac{1}{51200}$ molecule solution may be considered as the strength of the copper salts which will barely permit the roots to live.

A series of experiments similar to the above were also performed with seedlings of *Zea Mais* with the following results:

ZEA MAIS.

Copper salts.		$\frac{1}{6400}$ mol.	$\frac{1}{12800}$ mol.	$\frac{1}{25600}$ mol.	$\frac{1}{51200}$ mol.	$\frac{1}{102400}$ mol.
$CuSO_4$	1st 24 hours	....	....	1 mm	2.5 mm	16.5 mm
	2d 24 hours	....	....	.....	.....	7 "
$CuCl_2$	1st 24 hours	....	....	1 "	1.25 "	11.5 "
	2d 25 hours	....	....	.....	.....	7.5 "
$Cu(C_2H_3O_2)_2$	1, 24 h	....	....	.....	2 "	14 "
	2, 24 h	....	....	.....	.....	13.5 "

Now since the experiments with the acids showed that the seedlings of *Zea Mais* were able to withstand a greater amount it was thought that they would likewise withstand a greater amount of copper. For this reason only the first three dilutions shown

in the above table were used, but it was found that in all except the last no growth resulted, and in the $\frac{1}{25600}$ molecule solution only a very small increase in length was noted in the $CuSO_4$ and $CuCl_2$ and none in the $Cu(C_2H_3O_2)_2$. The roots at the end of the experiment were soft and flexible and showed a greenish color similar to the roots of *Pisum sativum* in the copper solutions. Two higher dilutions were then used; $\frac{1}{51200}$ gram-molecule, and $\frac{1}{102400}$ gram-molecule. In the first of these very slight growth occurred during the first twenty-four hours but no further growth afterwards, while in the $\frac{1}{102400}$ solution the growth was considerable during the entire period of the experiment. The $\frac{1}{102400}$ gram-molecule solution is to be considered as the one which will first allow the seedlings to grow. That the seedlings of *Zea Mais* should be less sensitive to acids and more sensitive to copper salts seems a little strange, but the experiments plainly show that this fact is true.

When $CuSO_4$ exists in dilute solution, it will dissociate to form $^{+}Cu^{+}$ ions and $^{-}SO_4^{-}$ ions, and at the degree of concentration of the solutions used the dissociation would be practically complete. Hence only copper-ions and $^{-}SO_4^{-}$ ions need be taken into consideration. Now it has already been shown in the case of H_2SO_4 that the SO_4^{-} ion is non-poisonous, at least in dilute solutions, so the Cu-ion is left to bring about the toxic action. $CuCl_2$, the second salt used, will dissociate to form $^{+}Cu^{+}$ ions and two Cl^{-} ions, and here also the dissociation will be practically complete in the solutions used, so that in this case we have to deal simply with $^{+}Cu^{+}$ ions and Cl^{-} ions. In the experiments with HCl it has been shown that the Cl ions are without effect, so that here also the toxic action must be due to the Cu ions. The next salt, $Cu(C_2H_3O_2)_2$ will dissociate to form $^{+}Cu^{+}$ ions and two $C_2H_3O_2^{-}$ ions, and here also the dissociation will be practically complete in the solutions used. Here then we have to deal simply with $^{+}Cu^{+}$ ions and $C_2H_3O_2$ ions. That the toxic action of this salt is due entirely to the Cu-ions can be clearly shown from the results obtained for the other copper salts. In the $CuSO_4$ and in the $CuCl_2$ the

toxic action was due to the Cu alone, the $^{-}SO_4^{-}$ ions and the Cl^{-} ions being without effect. Now in the case of the copper acetate if the $C_2H_3O_2^{-}$ was poisonous we should expect that the solution would kill the seedlings at a greater dilution, since it would have the combined action of H^{+} ions and $C_2H_3O_2^{-}$ ions, but the experiments show the killing point for the acetate to be the same as for the other salts. Hence we must conclude that the $C_2H_3O_2$ ion is without any poisonous effect at this dilution.

Nageli[12] has shown that copper by its mere presence in the water in which plants were growing was able to produce toxic effects, and Löw[13] has also shown that water which was distilled from a copper retort resulted injuriously to plants. True also found that it was impossible to use brass pins to fasten seedlings to a cork while growing in various solutions. That the poisonous effect was due to the Cu and Zn of the pins is shown by the fact that as soon as glass pins were substituted the plants grew without any difficulty. We commonly think of copper as being insoluble but in the cases mentioned above it is very certain that enough Cu-ions were formed in the solutions to produce the toxic action.

A better idea of the extremely small amount of copper necessary to kill the seedlings can be obtained by putting the results in a different form. The seedlings of *Pisum sativum* were killed by the $\frac{1}{25600}$ gram-molecule solution which is the same as one part of copper to 404,423 parts of water. The seedlings of *Zea Mais* were killed by the $\frac{1}{51200}$ gram-molecule solution, which is equivalent to one part of copper to 808,846 parts of water.

Before leaving the copper salts one other fact should be mentioned. At the end of the experiments some of the seedlings were transferred to distilled water to see if the roots would revive, but in no case would the main root grow. The seedling as a whole was not dead, but would continue to grow and produce secondary roots above the part of the root which had been killed,

[12] Denkschr. d. schweizerischen naturf. Ges. **33**: 1. 1893.

[13] Landw. Jahrb. **20**: 235. 1891.

so that when it has been stated that the seedling was killed only the root has been referred to.

5. Nickel and Cobalt.

A series of experiments were carried out with two nickel salts, $NiSO_4$ and $Ni(NO_3)_2$ and two cobalt salts, $CoSO_4$ and $Co(NO_3)_2$. The same two seedlings, *Pisum sativum* and *Zea Mais* were used. First as to the results obtained with the seedlings of *Pisum sativum*. Seedlings were placed in the dilutions shown in the following table and the growth recorded for periods of twenty-four hours.

PISUM SATIVUM.

Nickel and Cobalt.		$\frac{1}{6400}$ mol.	$\frac{1}{12800}$ mol.	$\frac{1}{25600}$ mol.	$\frac{1}{51200}$ mol.	$\frac{1}{102400}$ mol.
$NiSO_4$	1st 24 hours	1.25 mm	1.5 mm	1.75 mm	10 mm	13 mm
	2d 24 hours	..	..	..	6.5 "	5 "
$Ni(NO_3)_2$	1st 24 hours	1 "	.75 "	2.25 "	7 "	13.25 "
	2d 24 hours	..	..	..	..	11.75 "
$CoSO_4$	1st 24 hours	1 "	4 "	7.5 "	6.25 "	..
	2d 24 hours	..	..	3.5 "	5.75 "	..
$Co(NO_3)_2$	1st 24 hours	1 "	3 "	4.25 "	7.5 "	..
	2d 24 hours	..	..	1 "	4.75 "	..

First as to the nickel salts. In the $\frac{1}{6400}$, $\frac{1}{12800}$ and $\frac{1}{25600}$ gram-molecule solutions a small amount of growth was observed for the first twenty-four hours but no further growth resulted. In the $\frac{1}{51200}$ and $\frac{1}{102400}$ gram-molecule solutions considerable growth occurred for the entire period, except in the $\frac{1}{51200}$ nickel nitrate, in which solution the normal conditions were not fulfilled, since the plants suffered from a copious growth of bacteria. At the end of the experiment the roots which were killed were not soft and flabby as in the acid or copper poisoning, but were extremely rigid. The roots were so rigid and brittle that if the seedlings were dropped on the table they would snap in pieces almost like so much glass. For the cobalt salts growth was observed in the $\frac{1}{6400}$ and $\frac{1}{12800}$ gram-molecule solutions for the first twenty-four hours, but no growth afterwards, while in the two next weaker solutions growth continued for the entire period. The roots

which were killed by the cobalt solutions were also very brittle and rigid, the same as in the nickel solutions.

The results obtained for the seedlings of Zea Mais in the different solutions are given in the following table:

ZEA MAIS.

Nickel and Cobalt.	$\frac{1}{1600}$ mol.	$\frac{1}{3200}$ mol.	$\frac{1}{6400}$ mol.	$\frac{1}{12800}$ mol.	$\frac{1}{25600}$ mol.	$\frac{1}{51200}$ mol.
$NiSO_4$ 1st 24 hours	..	..	2 mm	3 mm	2.5 mm	11 mm
$NiSO_4$ 2d 24 hours	..	..	..	..	..	8 "
$Ni(NO_3)_2$ 1st 24 h'rs	..	..	..	5.5 "	4 "	17.5"
$Ni(NO_3)_2$ 2d 24 h'rs	..	..	..	..	..	16.5"
$CoSO_4$ 1st 24 hours	2.5 mm	1 mm	20.5 "	27 "	..	..
$CoSO_4$ 2d 24 hours	..	..	15 "	18 "	..	..
$Co(NO_3)_2$ 1st 24 h'rs	2 "	3.25"	22.5 "	19 "	..	..
$Co(NO_3)_2$ 2d 24 h'rs	..	..	19 "	34 "	..	..

The seedlings were set first in $\frac{1}{6400}$, $\frac{1}{12800}$, $\frac{1}{25600}$ and the $\frac{1}{51200}$ gram-molecule solution of $NiSO_4$. The first three showed growth for the first twenty-four hours but none afterwards, while those in the last solution grew for the entire period of the experiment. For the $Ni(NO_3)_2$ only the three solutions shown in the first table were used; in the first two growth occurred during the twenty-four hours, but none afterwards, so that the killing point for the nickel salts may be considered the $\frac{1}{25600}$ gram-molecule solution.

Reasoning from the results found for the *Pisum sativum* seedlings it was thought that the killing point for the cobalt salts might be found by the $\frac{1}{6400}$ and $\frac{1}{12800}$ gram-molecule solutions, but both of these dilutions gave a considerable growth for the entire forty-eight hours. Seedlings were then set in two stronger solutions, $\frac{1}{1600}$ and $\frac{1}{3200}$ gram-molecules. In these the roots grew for the first twenty-four hours, but no further growth had resulted at the second measurement. The killing point for the cobalt salts is thus placed at the $\frac{1}{3200}$ gram-molecule solution.

One point which must be noted in connection with all of the experiments with the nickel and cobalt salts is that in nearly every case a certain amount of growth resulted during the first

twenty-four hours of the experiments in which the roots were killed. The nickel and cobalt are quite poisonous, but the toxic effect is not felt by the plant as soon as in the case of acid or copper poisoning. The toxic action of nickel is seen to be greater than cobalt, by the results on both *Pisum sativum* and *Zea Mais*.

In the case of the $NiSO_4$ the dissociation will be into $^{+}Ni^{+}$ ions and $^{-}SO_4^{-}$ ions. The $CoSO_4$ will form $^{+}Co^{+}$ ions and $^{-}SO_4^{-}$ ions. At the dilution used for both of these salts the dissociation will be practically complete. It has already been shown that the SO_4^{-} ion is non-poisonous, so the $^{+}Ni^{+}$ ions and the $^{+}Co^{+}$ ions are left to bring about the toxic action.

The $Ni(NO_3)_2$ will form in dilute solution $^{+}Ni^{+}$ ions and two NO_3^{-} ions. The $Co(NO_3)_2$ will dissociate to form $^{+}Co^{+}$ ions and two NO_3^{-} ions. Here also the dissociation will be practically complete at the dilutions used, so that we have to deal simply with the resulting ions. The NO_3^{-} ion has been shown to be non-poisonous, so that in this case also the toxic action must be due to the $^{+}NO^{+}$ ions and to the $^{+}Co^{+}$ ions.

The small amount of ionic Ni and Co necessary to kill the roots is shown below:

PISUM SATIVUM.

Nickel—$\frac{1}{256\,00}$ mol.=1 part Ni to 435,374 H_2O.
Cobalt—$\frac{1}{128\,00}$ mol.=1 part Co to 217,687 H_2O.

ZEA MAIS.

Nickel—$\frac{1}{256\,00}$ mol.=1 part Ni to 435,374 H_2O.
Cobalt—$\frac{1}{32\,00}$ mol.=1 part Co to 54,421 H_2O.

Even in the case of the cobalt and *Zea Mais*, which shows the greatest concentration for poisoning, the amount of ionic Co is very small when compared with the amount of H_2O.

6. Silver Salts.

In testing the toxic action of silver two salts were used, silver sulfate and silver nitrate. For these experiments the same two seedlings were used. The results obtained for the seedlings of *Pisum sativum* are given in the following table:

PISUM SATIVUM.

Silver Salts.		$\frac{1}{51200}$ equiv.	$\frac{1}{102400}$ equiv.	$\frac{1}{204800}$ equiv.	$\frac{1}{409600}$ equiv.	$\frac{1}{819200}$ equiv.
Ag_2SO_4	1st 24 hours	..	..	8.25 mm	12.25 mm	11.25 mm
	2d 24 hours	..	..	11.75 "	8.5 "	14.25 "
$AgNO_3$	1st 24 hours	..	..	5.5 "	7.0 "	9.5 "
	2d 24 hours	..	..	4.5 "	7.5 "	10.5 "

The solutions here are expressed in equivalents of the toxic ion, since in the Ag_2SO_4 if we had the same fraction of a gram-molecule as in $AgNO_3$, the solution would contain twice as many silver ions. The dilutions were made as follows for Ag_2SO_4:

25cc of $\frac{1}{100}$ mol. to 400cc = $\frac{1}{1600}$ mol.
25cc of $\frac{1}{1600}$ mol. to 400cc = $\frac{1}{25600}$ mol. = $\frac{1}{128000}$ equiv.
100cc of $\frac{1}{25600}$ mol. to 400cc = $\frac{1}{102400}$ mol. = $\frac{1}{51200}$ equiv.
100cc of $\frac{1}{102400}$ mol. to 200cc = $\frac{1}{204800}$ mol. = $\frac{1}{102800}$ equiv., etc.

The dilutions for $AgNO_3$ were made as follows:

10cc of $\frac{1}{2}$ mol. to 1000cc = $\frac{1}{200}$ mol.
25cc of $\frac{1}{200}$ mol. to 200cc = $\frac{1}{1600}$ mol.
25cc of $\frac{1}{1600}$ mol. to 400cc = $\frac{1}{25600}$ mol.
100cc of $\frac{1}{25600}$ mol. to 200cc = $\frac{1}{51200}$ mol., etc.

In the first two dilutions no growth whatever occurred for the entire period and at the end of the experiment the roots were quite rigid, but not more so than in the ordinary seedlings grown under normal conditions. In the last three dilutions considerable growth was observed, so that the killing point for the silver salts is placed at $\frac{1}{102400}$ equivalents.

ZEA MAIS.

Silver Salts.		$\frac{1}{51200}$ equiv.	$\frac{1}{102400}$ equiv.	$\frac{1}{204800}$ equiv.
Ag_2SO_4	1st 24 hours..	1 mm	5 mm	17 mm
	2d 24 hours..	.....	...	12 "
$AgNO_3$	1st 24 hours..	1.5 mm	4 mm	11.25 "
	2d 24 hours..	.....	...	9.25 "

The results of the experiments for the seedlings of *Zea Mais* are given in the preceding table.

For the seedlings of *Zea Mais* it was only necessary to use three dilutions. In the first two of these the roots showed a slight growth during the first twenty-four hours, but no further growth was shown by the second measurement. In the $\frac{1}{204800}$ equiv. solution the growth continued for the entire period, so that here, as in the case of *Pisum sativum* experiments, the killing point is shown to be the $\frac{1}{102400}$ equiv. solution. Here also the roots which were killed remained quite rigid.

In Ag_2SO_4 the dissociation will be into two Ag^+ ions and $^-SO_4^-$ ions. The $AgNO_3$ will dissociate to form Ag^+ ions and $^-NO_3^-$ ions. It has already been shown in several cases that the $^-SO_4^-$ ions and NO_3^- ions are without any toxic action. Now since dissociation is practically complete in the dilutions used, only the ions are to be considered, and the non-poisonous character of the electro-negative ions leaves the Ag^+ ions to produce the toxic effect.

The extremely small amount of ionic silver necessary to kill the root may be expressed as follows:

Both seedlings killed by $\frac{1}{102400}$ eq.=1 part Ag to 948,148 H_2O. According to the above the silver ion is somewhat more poisonous than the copper ion.

9. Mercury.

Experiments were performed with the seedlings of *Pisum sativum* and *Zea Mais* and a single mercury salt, $HgCl_2$. The results of these experiments are given in the following table:

CORROSIVE SUBLIMATE.

$HgCl_2$		$\frac{1}{12800}$ mol.	$\frac{1}{25600}$ mol.	$\frac{1}{51200}$ mol.	$\frac{1}{102400}$ mol.	$\frac{1}{204800}$ mol.	$\frac{1}{409600}$ mol.
Pisum sativum	1st 24 hrs	...	2mm	2.25mm	3.75mm	5.75mm	4.5mm
	2d 24 hrs	...	....	.....	.....	7.25 "	4 "
Zea Mais	1st 24 hrs	2mm	7.5mm	19mm	14.5mm	.....	....
	2d 24 hrs	...	10	28	10.5 "	.....	....

The dilutions were made from the $\frac{1}{100}$ molecule solution the same as for the Ag_2SO_4. In the first three dilutions used for the seedlings of *Pisum sativum* a growth of a few millimeters occurred during the first twenty-four hours, but the second measurement showed no further growth. Those roots which were killed remained rigid, but were somewhat discolored at the end of the experiment. In the last two dilutions growth continued throughout the experiment, so that the killing point for the seedlings of *Pisum sativum* is shown to be the $\frac{1}{102400}$ gram-molecule solution. For the seedlings of *Zea Mais* the $\frac{1}{51200}$ and $\frac{1}{102400}$ gram-molecule was first used, but in both of these growth resulted, so two more concentrated solutions were used, which showed the killing point to be the $\frac{1}{12800}$ gram-molecule solution. This, then, shows that the seedlings of *Zea Mais* are able to withstand a much greater amount of mercury than the *Pisum* seedlings.

The $HgCl_2$ will dissociate to form $^{+}Hg^{+}$ ions and two Cl^{-} ions, and at the degree of concentration used the dissociation will be practically complete, so we have to deal simply with $^{+}Hg^{+}$ ions and Cl^{-} ions. The Cl^{-} ions have been shown to be non-poisonous, so the toxic effect must be due to the ionic mercury. The amount of ionic mercury necessary to kill the plants may be expressed as follows:

Pisum sativum: seedlings killed by $\frac{1}{102400}$ mol. = 1 part Hg to 510,978 H_2O.

Zea Mais: seedlings killed by $\frac{1}{12800}$ mol. = 1 part Hg to 63,872 H_2O.

8. Potassium Cyanide.

The effect of CN on the roots of *Pisum sativum* and *Zea Mais* seedlings was tested by solutions of KCN. The results obtained with different solutions are shown in the table on next page.

For the *P. sativum* seedlings no growth whatever occurred in the $\frac{1}{1600}$ and $\frac{1}{3200}$ gram-molecule solutions, while in the $\frac{1}{6400}$ gram-molecule solution growth was noted during the first twenty-four hours but no further growth for the second measurement.

In all of the solutions by which growth was inhibited the roots were quite rigid at the end of the experiment. In the last two dilutions growth was noted for the entire period, so the killing point is shown to be the $\frac{1}{6400}$ gram-molecule solution. The seedlings of *Zea Mais* were started in the $\frac{1}{3200}$ gram-molecule solution which completely inhibited the growth, while the two next dilutions allowed growth to continue, so that here the killing point is shown to be the $\frac{1}{3200}$ gram-molecule solution showing the *Zea Mais* to be somewhat more resistant than the *P. sativum*.

POTASSIUM CYANIDE.

KCN		$\frac{1}{1600}$ mol.	$\frac{1}{3200}$ mol.	$\frac{1}{6400}$ mol.	$\frac{1}{12800}$ mol.	$\frac{1}{25600}$ mol.
P. sativum	1st 24 hours	....	....	1.25 mm	3 mm	2.5 mm
	2d 24 hours	....	....	....	2 "	3 "
Z. Mais..	1st 24 hours		....	2.5 "	3 "	
	2d 24 hours		....	7.5 "	6 "	

The KCN will dissociate to form K^+ ions and CN^- ions, and the dissociation is nearly complete. Since we are dealing here with a potassium salt of HCN and quite dilute solutions, the amount of undissociated KCN will not be very great. At the dilutions used the K^+ ion would not produce any toxic action, so the poisonous quality of the KCN solutions must be due principally to the CN^- ion and to a slight extent to the undissociated KCN, the effect of which will decrease as the dilutions become greater.

9. Potassium Ferro- and Ferri-cyanides.

Experiments were performed with the seedlings of *P. sativum* and *Zea Mais* and potassium ferro- and potassium ferri-cyanide.

In the $\frac{1}{25}$ and $\frac{1}{50}$ gram-molecule solutions of $K_4Fe(CN)_6$ the roots were killed, no growth whatever resulting. In $\frac{1}{100}$ molecule solution growth was observed during the first twenty-four hours, but none afterward. In the $\frac{1}{200}$ and $\frac{1}{400}$ gram-molecule solution growth continued for the entire period. In the $K_3Fe(CN)_6$, the seedlings were set first in the $\frac{1}{100}$ molecule solution in which no

growth occurred. The two next weaker solutions allowed the seedlings to grow. The killing point may be placed, then, at $\frac{1}{100}$ gram-molecule. Those roots which were killed remained rigid, but were somewhat discolored. In the weaker solutions which did not entirely inhibit the growth a retardation of the growth is apparent.

The results of the experiments with *P. sativum* are given in the following table:

PISUM SATIVUM.

CYANIDES.		$\frac{1}{25}$ mol.	$\frac{1}{50}$ mol.	$\frac{1}{100}$ mol.	$\frac{1}{200}$ mol.	$\frac{1}{400}$ mol.
$K_4Fe(CN)_6$	1st 24 hours	....	....	1 mm	2.25 mm	7.5 mm
	2d 24 hours	....	....	....	.75 "	5 "
$K_3Fe(CN)_6$	1st 24 hours			....	5 "	7 "
	2d 24 hours			....	3 "	4 "

The results of the experiments with *Zea Mais* are given in the following table:

ZEA MAIS.

CYANIDES.		$\frac{1}{25}$ mol.	$\frac{1}{50}$ mol.	$\frac{1}{100}$ mol.	$\frac{1}{200}$ mol.
$K_4Fe(CN)_6$	1st 24 hours	....	1.5 mm	2.5 mm	6.75 mm
	2d 24 hours	....	....	....	7.25 "
$K_3Fe(CN)_6$	1st 24 hours	....	1.5 "	8.25 "	15.25 "
	2d 24 hours	....	....	....	3.75 "

In both of the salts no growth occurred in the $\frac{1}{25}$ molecule solution. In the $\frac{1}{50}$ mol. and $\frac{1}{100}$ mol. solutions growth was noted for the first twenty-four hours but none afterwards. The $\frac{1}{200}$ mol. solution showed growth for the entire period, so that here as in the case of the *P. sativum* seedlings the killing point is the $\frac{1}{100}$ gram-molecule solution.

In the case of both salts the dissociation will be in the form of K^+ ions and $Fe(CN)^-$ ions, the only difference being the fact that whereas $K_4Fe(CN)_6$ solutions contain four K ions the $K_3Fe(CN)_6$ solutions contain three K ions. For the dilutions used the dissociation is not complete.

For the $K_4Fe(CN)_6$ at 18° C. and of $\frac{1}{32}$ gram-molecule strength about $\frac{15}{26}$ of the molecules will be broken up, and for a $\frac{1}{512}$ gram-molecule solution about $\frac{23}{26}$ of the entire number of molecules will be split up. For the $K_3Fe(CN)_6$ at 18° C. and of $\frac{1}{96}$ gram-molecule strength, about $\frac{18}{23}$ of the total number of molecules will be dissociated.[14] The solutions used will then contain a certain number of undissociated molecules besides the K^+ ions and the $Fe(CN)_6^-$ ions. The K^+ ions have already been mentioned as non-poisonous at these dilutions, so the toxic action must be referred to the $Fe(CN)_6^-$ ions and to some extent to the undissociated molecules.

The much greater strength of solutions which the seedlings are able to withstand in the above experiments over that for the KCN, show that the CN has lost its toxic action to a great extent by combining with the Fe to form the $Fe(CN)_6$ ion.

The roots of *P. sativum* were killed by the $\frac{1}{6400}$ KCN, while it took $\frac{1}{100}$ potassium ferro- or ferri-cyanide. The molecule of the potassium ferro- or ferri-cyanides contained six times as much cyanogen, hence it required 384 times as much cyanogen in the form of the $Fe(CN)_6$ ion to produce the same effect. The roots of *Zea Mais* were killed by the $\frac{1}{3200}$ KCN, while it took $\frac{1}{100}$ gram-molecule of $K_4Fe(CN)_6$ or $K_3Fe(CN)_6$ to produce the same effect, or 192 times as much CN in the form of the $Fe(CN)_6^-$ ion.

10. Silver Nitrate + 3KCN.

Some experiments were also performed to test the action of $AgNO_3 + KCN$ upon the growth of the same seedlings as had been used before. The results are given below:

		$\frac{1}{12800}$ mol.	$\frac{1}{25600}$ mol.	$\frac{1}{51200}$ mol.	$\frac{1}{102400}$ mol.	$\frac{1}{204800}$ mol.
P. sativum	1st 24 hrs...	2.5mm	3mm	3mm	6mm	2.5mm
	2d 24 hrs...	....	.....	3.5	2.5	4
Z. Mais	1st 24 hrs...	1.5	3.25	6	8.25	....
	2d 24 hrs...	....	3.25	2	5.25	....

[14] Ostwald, Chemische Energie 739.

For the *P. sativum* seedlings growth occurred in the first two dilutions for the first twenty-four hours only. In the next dilutions growth continued for the entire period. For *Zea Mais* growth was noted in the first dilution for the first twenty-four hours, but not afterwards, while in the following dilutions growth continued for the entire period. Those roots which were killed remained rigid.

The solutions were mixed upon Ag as a base, and the following action will show what the solutions contain:

$$AgNO_3 + 3KCN = KNO_3 + K(AgCN_2) + KCN.$$

At the dilutions used the KNO_3 is without effect. The $K(AgCN_2)$ will dissociate to form K^+ ions and $AgCN_2^-$ ions; the KCN to K^+ ions and CN^- ions.

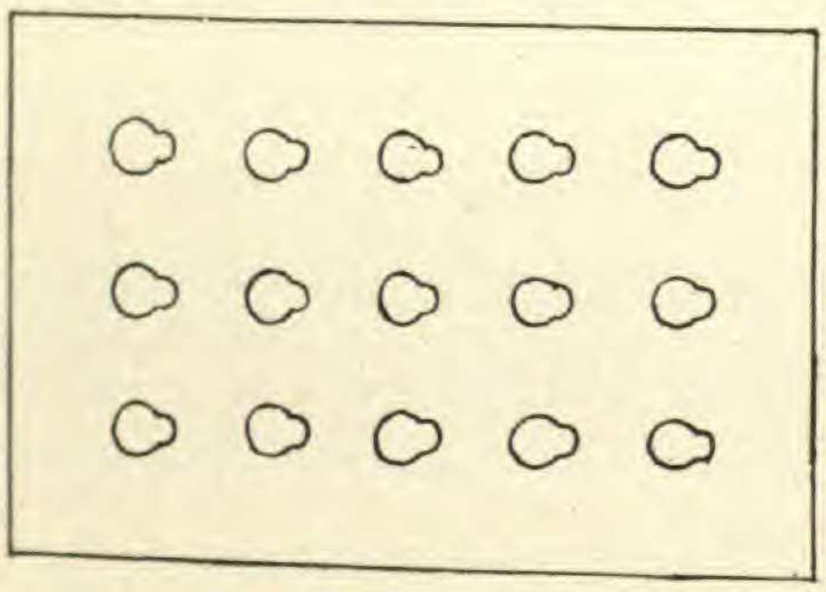
The above experiments show that more silver is required to produce poisoning when present in the form of $AgCN_2^-$ ions, than when present as the simple Ag ion: four times as much for the *P. sativum* seedlings and eight times as much for *Zea Mais* seedlings.

A comparison of the results obtained by myself with those of True and Kahlenberg[15] is given in the following table. The dilutions given just allowed growth.

Name of Compound	P. sativum	Z. Mais	L. albus.
HCl	1/12800 eq.	1/3200 eq.	1/6400 eq.
H_2SO_4	" "	" "	" "
HNO_3	" "	" "	" "
HBr	" "	" "	" "
$C_2H_4O_2$	"	1/400 "	1/1600 "
$CuCl_2$	1/3200 "	1/102400 "	1/25600 "
$CuSO_4$	1/51200 "	" "	" "
$Cu(C_2H_3O_2)_2$	" "	" "	" "
$NiSO_4$	" "	" "	" "
$Ni(NO_3)_2$	1/51200 "	1/51200 "	1/25600 "
$CoSO_4$	" "	" "	..... "
$Co(NO_3)_2$	1/25600 "	1/6400 "	1/12800 "
$AgNO_3$	" "	" "	" "
Ag_2SO_4	1/204800 "	1/204800 "	1/204800 "
$HgCl_2$	1/204800 "	" "	" "
KCN	1/204800 "	1/51200 "	1/12800 "
$K_4Fe(CN)_6$	1/12800 "	1/6400 "	1/6400 "
$K_3Fe(CN)_6$	1/200 "	1/200 "	1/200 "

[15] BOT. GAZ. 22: 81. 1896.

1.



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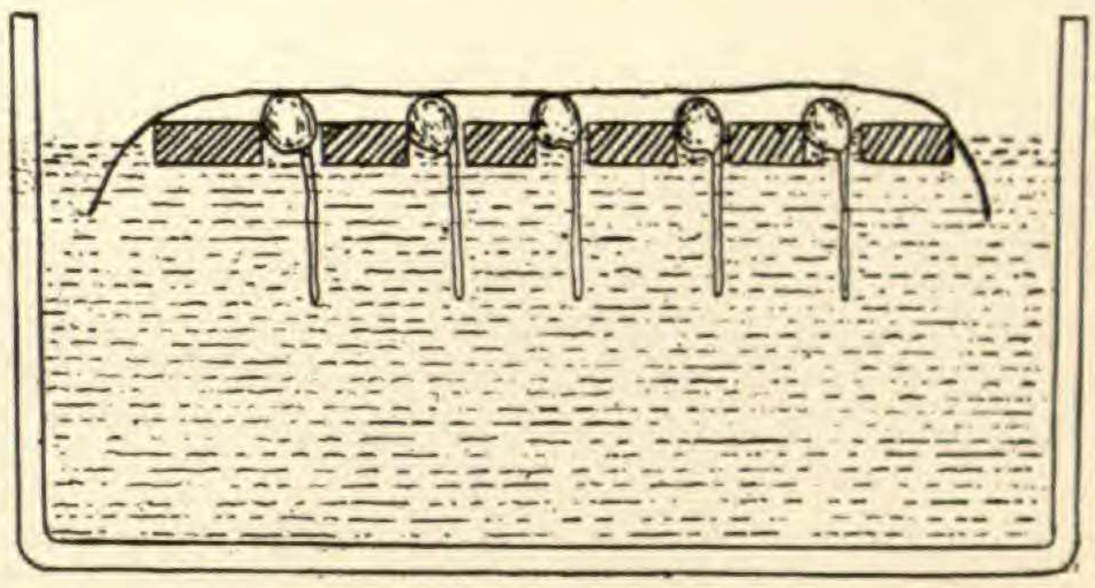

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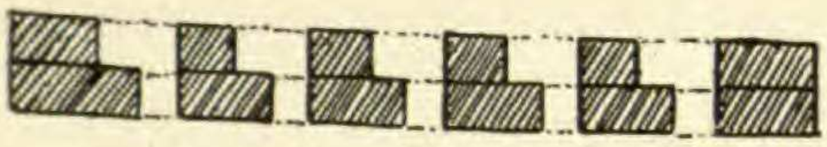

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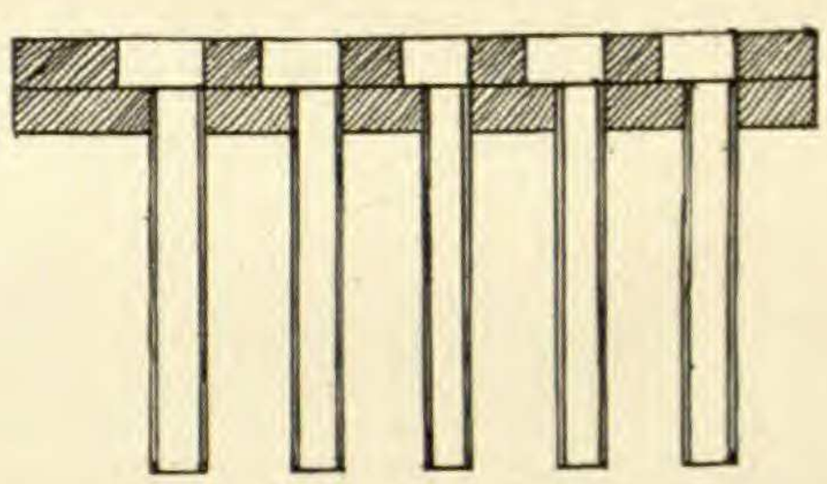

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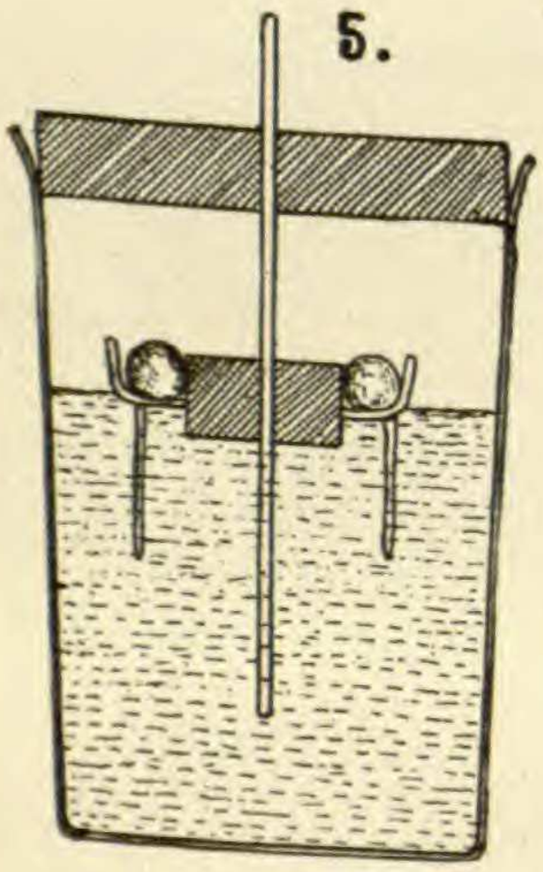

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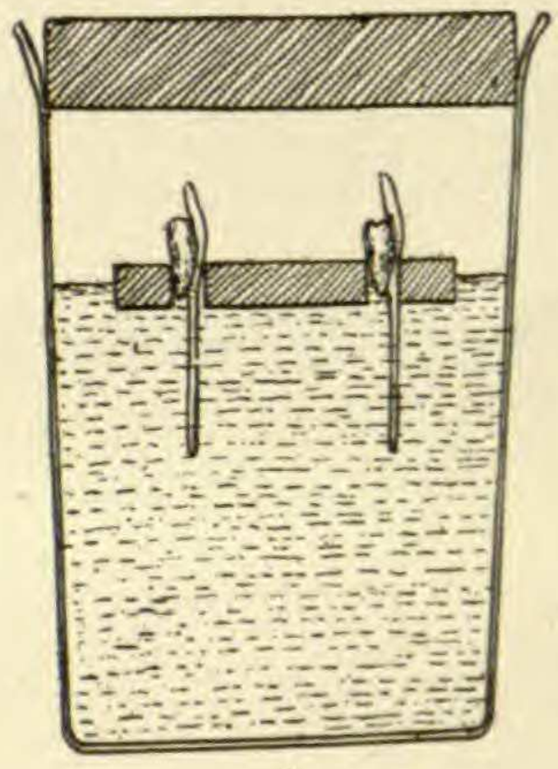

HEALD on EFFECT of DILUTE SOLUTIONS.